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Impact of Traditional and Modern Dairy Practices on Clean Milk Production and Mastitis

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Milk is a very important and highly nutritious diet for human beings. It is normally a sterile product when secreted from the udder of a healthy dairy animal. Natural milk is a complete food enriched with a variety of nutrients (protein, lipid, lactose, minerals etc.). It is an ideal medium and highly susceptible to microbial spoilage. If contaminated with microbes, it may cause severe and deadly milk borne infectious diseases, i.e., salmonellosis, tuberculosis, brucellosis and clostridial infections etc. to humans. Farm fresh milk is needed to be protected against all possible sources of contamination. The present study was conducted to determine the various factors involved in production of good quality milk. Various factors that contaminate the milk include cleanliness of udder, animal health, cleanliness of utensils, personnel hygiene, environmental hygiene and feed quality. This study was carried out on 5 dairy herds. These herds were ssituated in different localities in Faisalabad. Isolation of microorganisms, Somatic Cell Counts (SCC) and Colony Count (CC) were carried out on these herds to determine the milk quality. To this end, herds were screened by Surf Field Mastitis Test (SFMT) for the detection of subclinical mastitis. All the positive milk samples were submitted to Mastitis Research Lab, University of Agriculture Faisalabad, for the detection of microorganisms and CC. Moreover, somatic cell count was performed on the milk of SFMT positive and SFMT negative animals. A total of 10 animals on each farm were selected randomly and divided into 3 groups *viz*. non-washed udder (n=10), washed udder (n=10) with simple water and clean with towel and disinfected udder (n=10) and clean with towel. Results indicated the d**ecreas**e in SCC and CC in the disinfected and clean with towel udder significantly **(P<0.01)**. However, SCC and CC were increased significantly **(**P<0.05) in control groups. All in all, by applying udder washing with disinfectant and clean with towel warranted the clean milk production.

Keywords: Milk quality, somatic cell count, colony count, mastitis, dairy farm management.

INTRODUCTION

Asian dairy consumption and production are world leading. In recent decades, milk and dairy products satisfied the regional demand. Milk consumption is at an all-time high, over double the global average, and will climb to almost 320 million tonnes by 2021 (Ahuja *et al.,* 2014). Pakistan's population and milk consumption are rising. Pakistan has 27.3 million buffaloes and 29.6 million cattle. Faisalabad's urban and peri-urban areas have 2% buffalo and 0.65% cattle (Qureshi *et al.,* 2003). Milk output falls short of population needs. Milk farmers spend a lot on disease, reproductive problems, and mastitis (Ahuja *et al.,* 2014). Subclinical mastitis (SCM) plagues dairy businesses (Kader *et al.,* 2002). Subclinical mastitis triples losses. Not only milk production is falling. However, compositional changes damage the dairy processing (Khan *et al.,* 2005). The National Mastitis Council (Bramley, 1996) reports 80% milk loss from subclinical mastitis. Subclinically infected animals spread disease (Muhammad *et al.,* 2010). Mastitis therapy releases antibiotic residues that impact consumers and lowers milk quality. Clinical mastitis affects 17% of cattle and 21% of buffalo (Bilal *et al.,* 2004). Most clinical mastitis in Pakistan is bacterial and fungal. Also, yeasts can induce mastitis. Bacteria are the most prevalent mastitis pathogen. *Staphylococcus aureus* is the most common bacteria in dairy

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cows and buffaloes (Hussain *et al.,* 2007). *Staphylococcus* 29%, *E. coli* 16%, *Streptococcus* 7.55%, *Pseudomonas* 13.30%, *Salmonella* 7.20%, *Bacillus* 12.42%, *Enterococcus* 3.17%, *Coryneacterium* 6.64%, and *S. aureus* 38% are other mastitis pathogens (Ali *et al.,* 2011). Dairy animals suffer economically from mastitis (Chishty *et al.,* 2007). It affects milk quality and supply. The substantial milk output drop alters its physico-chemical properties. The pH of milk rose from 6.88 to 6.57±0.021. Blood component permeability elevates gland tissue pH (Rao, 1990). Reduced udder epithelial cell production and secretary activities lower milk fat (Goncalves *et al.,* 2018). Pre-milking photolytic enzyme activity degrades udder milk protein, reducing protein %. Mastitis was the most hazardous disease in field surveys (Ajmal, 1990). A small herd requires hand-milking. Milker's hand can spread mastitis (Contreras *et al.,* 2007).

More infections occur when milk is used as a "lubricant" during milking. Clean milk production requires udder maintenance and cleanliness. Some farmers cleanse the udder before milking, but most lubricate with milk. Mastitis is caused by udder water bacteria entering the streak canal. Somatic cell count (SCC) increases during milking, reducing milk supply and quality. Intramammary infection and teat bacteria are positively correlated. The deadlier *Staphylococcus aureus* causes nursing cow mastitis. *Staphylococcus aureus* colonization on teat skin increases clinical mastitis. Udder washing and towel drying minimize milk germs. This reduces winter milk bacterial contamination best. Some dairy producers dip teats pre- and post-milking with antiseptics such as Dettol, Povidone-iodine, hypochlorite solutions, ALBSA, chlorides, Chlorhexidinum, phenolic, and alcoholic compounds instead of washing and drying the udder and teat canal. These disinfectants all lower SCC and bacteria (Gleeson *et al.,* 2009). Hide faeces may contain pathogenic enteric bacteria, causing food-borne illness. Udder cleanliness affects milk supply, thermoregulation, and health (Reneau *et al.,* 2003; Ruud, 2011). Manure-etched skin can cause subclinical illnesses and pain. Faeces, muck, dust, and vegetative matter from animal faeces and animal-to-animal or environment-to-animal contamination discolour hides. Diarrhea caused soiling on the body partes of cow. Diarrhea and loose manure might result from diseases or high-protein, low-fiber diets (Hughes, 2001).

Udder infections require post-milking dipping. Many microorganisms, especially Escherichia coli, live in soil. These viruses increase summer mastitis. Transmission is highest during milking and post-milking. Because the teat orifice remains open 30 minutes after milking. Nearly 75% of *Escherichia-Coli* infections induce inappropriate milk, udder oedema, high temperatures, and appetite loss (Cinar *et al.,* 2015).

After milking, cows' teats must be dipped in an effective germicide to prevent intra-mammary infections throughout lactation (Contreras *et al.,* 2007).

This study examined milk quality, clean milk production factors, and mastitis prevalence. The criteria of evaluation include:

- To compare traditional milking practices with modern dairy practices
- To evaluate the milk quality through somatic cell count (SCC).
- To calculate somatic cell count (SCC) in traditional milking system
- To evaluate somatic cell count (SCC) by applying Modern Dairy Practices in herd
- To determine colony count in raw milk

MATERIALS AND METHODS

***Source of milk samples*:** The survey covered Faisalabad's urban and peri-urban livestock holdings. This study examined theses six Roads Sargodha, Narowala, Samunderi, Satyana, Jhang, and Shiekhupora roads. Roads are further classified into three kinds. 1. Small-scale semi-commercial (n=10). 2. Small commercial farm (10-20). 3. Large-scale commercial farm ($n \geq 20$). Animal management, housing, nutrition, milking, and clinical examination data were recorded on Performas. SFMT screened each herd (Muhammad *et al.,* 2010). Each herd had two sub-groups positive mastitis and mastitis-free animals. Mastitis-negative samples were divided into three groups. Composite milk samples n=10 was obtained from non-washed, washed, and antiseptically treated udders. Somatic cell and colony counts are calculated in the University of Agriculture Faisalabad Mastitis Research Lab Department of Clinical Medicine and Surgery.

***Base line survey*:** A Faisalabad-wide baseline survey was done. Surf Field Mastitis Test screened the complete herd (Muhammad *et al.,* 2010). All factors that affect clean milk production and mastitis were documented, including housing, nutrition (feed quality, feeding techniques, feeding plan), shed cleanliness, animal, worker, equipment, and milk parlor hygiene. Further, clinical positive and negative milk samples were obtained separately and submitted to Mastitis Research Lab, University of Agriculture Faisalabad, pursuant to the National Mastitis Council, Inc. USA (1990).

***Collection of clinical positive milk samples*:** The samples were taken from 64 subclinical and 35 clinical positive mastitis milk samples screened by SFMT (Muhammad *et al.,* 2010) of nursing cows and buffaloes of the aforesaid dairy farms according to National Mastitis Council, Inc. USA (1990). Using a Platinum inoculation loop, 0.01 ml of milk was placed onto blood agar (5% defibrinated sheep blood) and MacConkey agar plates. Inoculated blood and MacConkey agar plates were incubated at 37°C in the incubator. Gramme staining and other tests found these species at the genus level after 24 hours of growth on plates: *Escherichia coli*, *Staph. Aureus*, *Streptococcus* etc.

***Collection of composite milk samples for SCC and colony count*:** Following Surf Field Mastitis Test screening of the herd (Muhammad *et al.,* 2010). All SFMT-negative animals were divided into three categories. Milk was taken from each animal's milking pail in a vacutainer (National Mastitis Council, Inc. USA, 1999). Somatic cell and colony counts are calculated in the University of Agriculture Faisalabad Mastitis Research Lab Department of Clinical Medicine and Surgery.

***Estimation of sampling size*:** This calculation was made by according to (Muhammad *et al.,* 2010). The Confidence Interval was 95%.

$n=1.96^2(P_{exp})\ (1-P_{exp})/d^2$

N= required sample size; P_{exp} =Expected Prevalence; d = desired absolute precision

Sample Size adjustment

$N_{adj=}\ N\times n'/N+n'$

N = size of Population; n =sample size based on infinite population

***Preparation of milk film*:** Milk samples were properly mixed to distribute cells evenly. Three minutes' dry samples and prevent air bubbles. Using a permanent marker and card template, the microscopic glass slide was marked 1cm. Glass slide was placed on a clean, flat surface. A 0.01 ml milk sample was put on the center of the glass slide opposite the specified region with a micro-dispenser. The milk sample was uniformly distributed in the indicated area. With each fresh micro-dispenser tip. For five minutes, the dried milk film was soaked in xylene to de-fat. Newman's Lambert stain-stained dried milk film for 30 seconds (Schalm *et al.,* 1971).

***Staining*:** The dried milk slide is placed in xylene for 5 minutes to de-fat the milk sample. Dried the glass slide in the air then placed it on the staining stand with the help of a dropper an adequate amount of Newman's Lambert stain was emptied on milk film. The dried stained film was then washed with normal saline and kept in the beaker to flush the extra stain (Hussain, 2007).

***Cell counting method*:** Milk film was observed under a microscope under an oil immersion lens 100X. Fifty fields were counted by moving slides in zig-zag manners and the somatic cells in each field. The average number of cells was calculated and multiplied by a working factor of 40000 (Schalm *et al.,* 1971).

***Preparation of milk sample*:** The milk sample (1 ml) was mixed thoroughly to get a uniform distribution of cells. The samples were permitted to stand for five minutes to avoid air bubbles. Ten test tubes were taken with (9ml) normal saline in each tube. The milk sample (1.0ml) was added in the first tube and mixed gently to obtain a uniform dilution of microbes in a test tube. One ml sample was drawn from the first tube and then transferred to the second tube and was mixed gently**.** Then a further one ml sample was taken from the second tube and transferred to the third test tube and the same procedure was done up to 10^{th} test tube (Desmasures *et al.,* 1997).

***Procedure of dilution of sample*:** There are 10 test tubes having 9 ml distilled water. Then add 1 ml milk sample in the first tube. This tube contains a total of 10 ml of solution, 1 part milk sample, and 9 parts distilled water. Simply shake the sample gently and then take a sample from the first tube of 1 ml then pour it into the second tube, we have created a 1:100 dilution similarly shake the sample gently transfer it to the next test tube, and so on. This process is carried out until desired dilution factors are met when there were proper dilutions met, and then the contents of seven valves are used on the spreading of plates.

***Dilution of milk sample*:** One ml milk sample was used in the test tubes having 9 ml distilled water and labeled as 1:10. The vortex mixer was used to mix the tube contents at the end of each table. A pipette was used to transfer a milk sample of 1ml from the 1:10 tube to the next sterile tube, labeled as 1:100. The procedure continued, making transfers until 1:100 and 1:10000 dilutions were made. Then spread the volume of 0.01 ml with a micro dispenser across the surface of the plate containing nutrient media. Spread the volume of milk on a petri plate with a clean glass rod. Continued the making plates in this fashion from each of the dilution tubes until they have created four plates: 1:20, 1:200, 1:2000, and 1:20000. Place the plates in a rack inverted to be incubated (Desmasures *et al.,* 1997).

***Colony Counting Method* (Desmasures *et al.,* 1997)**

CFu /ml = (no. of colonies x dilution factor) / volume of the culture plate

$= 200\times10^7/0.01\text{ml} = 2.0\times10^7$ cfu/ml

***Data analysis*:** The collected data has been analyzed using the chi-square test through descriptive analysis. The relationship between different variables is determined through Pearson's correlation coefficient in SPSS (18.0) (Vyas *et al.,* 2006). Data was analyzed by using a factor Completely Randomized Design (CRD) and a comparison of means was done using Tukey's test at a 5% level of significance. Two sample t-tests were performed for comparison between washings (non-washed vs washed).

RESULTS

Baseline survey analysis: About 29% of farms had permanent dairy sheds, whereas 110 (61% had temporary ones). Milk revenues and farm expenses differed significantly ($P>0.01$) between semi-commercial and commercial farms. Traditional sheds were employed by 66% of houses (136). But just 25% have sophisticated scientific dairy farm design. There were significant variations ($P\geq0.05$) in farm design, drainage system, and shed cleaning frequency between semi-commercial and commercial farms. Despite 46% of farms having brick flooring, ~70% had cracks and fissures, allowing bug and tick hiding places. Semi-commercial and commercial

farms had similar factors. Pits and uneven surfaces formed permanent urine, wastewater, and slurry storage on 33% of these farms' floors. Urine, wastewater, and air pollutants are collected on 79% of farms without drainage. Most dairy animals (81%) had dirty rear quarters and udders with dried manure, and these farms did not cut hair (83%), trim hooves (74%), wash udders before milking (59%), or dip teats (n=152, 84%) after milk Commercial farms had better body cleanliness, udder washing before milking, hair clipping, foot trimming, and washing than semi-commercial farms (P>0.05).

Table 1. Percentage (%) of clean hind quarter of the animal.

Parameters	Frequency	Percent (%)
More dirty	53	29.4
Moderate dirty	46	25.6
Clean	52	28.9
Very Clean	29	16.1
Total	180	100.0

Only 7% of farms cleaned daily. On 19% of farms, cleaning occurred weekly. Cleaning occurred biweekly on 46% of farms. Air quality 16% of farms were clean, 40% adequate, and 32% unclean and smelly. This is also why milk smells like feces since it absorbs well. Disinfection was done on 19% of farms. All disinfection was not done on 76% of farms. Teat dipping and tick control were absent. Flies are the main cause of farm infections and diseases. Only 7% of farms had fly control techniques, while 87% had flies. Similar to tick prevalence, only 20% of farmers used tick control. On farms, all insects were 70% prevalent. Only 23% of farms were insect controlled. These factors were similar on semi-commercial and commercial farms. Chemical and injectable tick and pest control agents exist. About 60% of farmers utilized burning to control insects, 23% used spray, and 7% used manual methods. 2% used others' methods. Farms had varying tick control intervals. Only 6% of farms have weekly control measures, Fortnightly at 40% farms. Few farmers used bedding. Only 24% of farms changed bedding material, while 76% did not.

Table 2. Percentage (%) cleanliness of animal body condition.

Description	Frequency	Percentage (%)
Very Clean	5	2.8
Clean	37	20.6
Dirty	93	51.7
Very Dirty	45	25.0
Total	180	100.0

According to Table 2, animal bodies were extremely dirty (25%), 51% dirty, 20% clean, and 2.8% very clean. There were 27% farms with feces on the animal, 24% less contaminated, and 14% clean. Udder hygiene is also vital for clean milk production. Clean udders were found in 1% of animals. In 49% of animals, the udders were filthy. Only 16% of farms have evident animal grooming, 23% of farmers trimmed udder hair. Feces adhered to animal skin hairs during milking. Milk contamination was largely caused by that. 77% of farms didn't trim udder or hooves. Small semi-commercial and commercial farms had issues with animal hindquarters cleanliness. Only 2.8% of farms had sparkling udder and hindquarters after milking Nearly 42% of farms washed their udders while at 58% of farms, udder were not washed. Farm-level washing was 90% water, 8% water, and disinfectant. 50% of farms had teat damage. Injuries included 12% wounds, 15% scars, 29% lacerations, and 36%. There was a substantial difference (P<0.05) between pre- and post-dipping in commercial and non-commercial farms. Teat dipping was only done on 14% of farms with 15.6% monthly mastitis cases.

Milk hygiene conditions differed significantly (P<0.05) between commercial and non-commercial farms for workers practicing milking. Worker clothes were too unclean on 33% of farms, 55% of farms, and 6.7% of farms. There was a substantial difference (P<0.05) in milk microbiological load when workers washed their hands before milking as they were dirty

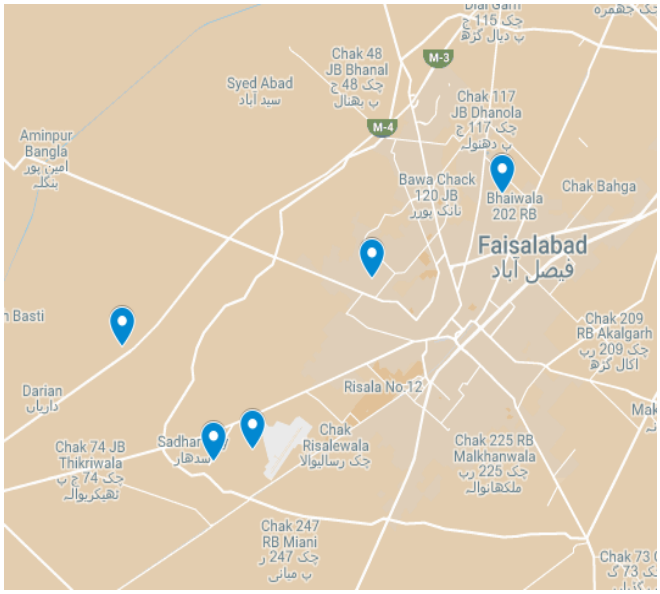
It was found that 49% of treated animal milk was sold with regular milk. Systemic illness or mastitis affected 33% of nursing animals. Few animals were healthy, 7% very well, 16% good, 44% bad, and 27% extremely poor.

The hands of 23% of workers were uninjured. Cuts, wounds, and lacerations affected 72%. Handwashing and disinfection before milking were crucial, however, only 16% of workers did. While 66% of workers covered their heads and started milking without washing their hands or using disinfectants. Mastitis prevalence varied greatly on farms. Where full-hand milking and knuckling were used. Full manual milking, 40% knuckling, and 30% striping were done on 23% farms. Milk hygiene and quality differed with clean milking pans. Only 4% of farms have separate milking areas. Milking was done in the herd on unclean floors at 90% of farms. Nearly 29% of farms used calf and 54% used oxytocin for milk let down. Utensils were cleaned 39% daily, 28% weekly, and 24% fortnightly. Steel 32%, plastic 36%, and storage tank 26%. Equipment was cleaned with 43% water, 19% disinfectant, and 31% sand. Only 8% of farmer’s dry equipment before bucket milking; 85% do not. Covering the milking tank was crucial. Only 23% of farmers cover the milking tank, while 70% do not. At farm level, 15% milk was dusty, 41% fecal, and 35% hairy. Milk smelled 7% bad, 10% feces, and 43% normal. On all farms, 74% of workers were uneducated and 19% were trained in hygiene.

Table 3. Percentage (%) of cleanliness of udder of animals before milking.

Description	Frequency	Percentage
Very good	18	10.1
Good	4	2.2
Moderate	28	15.6
Poor	91	50.8
Very Poor	38	21.2
Total	179	100.0

***Prevalence of mastitis in research area*:** Total 180 milking animals (n=180) include Zam Zam Dairy Farm (25 cows, 45 buffaloes), Irfan Dairy Farm Chak No. 241 RB Jhang Road (50 cows, 80 buffaloes), Nazir Dairy Farm (n=20 cows, 15 buffaloes), Bilal Dairy Farm (n=32 cows, 28 buffaloes), and Arz-e-Pak Dairy Farm (n=32 cows, 28 buffaloes). Zam Zam Dairy Farm at Sheikhupora Road has 21% subclinical mastitis (n=15 out of 70 animals), including 14% (n=10) in cows and 28.57% in buffaloes. The prevalence of subclinical mastitis at Irfan Dairy Farm was 30.76% (n=40 out of 130 animals), with 19.21% in buffaloes (n=25) and 12.30% in cows. Subclinical mastitis was 25.71% (n=9 out 35 animals) in Nazir dairy farm, comprising 20% (n=7) in cows and 11.4% (n=4) in buffaloes. Subclinical mastitis was found in 53.33% (32 of 60 animals) at Bilal Dairy Farm, including 10% (n=6) in cows and 21.66% (n=13) in buffaloes. Arz-e-Pak Dairy Farms had 38.5 % (31 out of 82 animals) subclinical mastitis, including 27.5 % (n=22) in cows and 20% (n=16) in buffaloes.



***Frequency of different mastitis pathogens in clinically mastitis milk samples submitted to University of Agriculture Faisalabad*:** On microbiological Examination of Surf Field Mastitis Test, positive milk samples from five dairy farms and 22 clinical mastitis milk samples submitted to Mastitis Research Lab, University of Agriculture Faisalabad, 96 isolates of 5 genera were isolated. No growth was found in 10 milk samples. Fifty-three of 96 isolates (59.81%) were gram-positive, catalase-positive s*taphylococci*. *S. Aureus* isolates were identified from 43 (67.18%) *Staphylococcal* isolates that produced coagulase at 4 hours. The *S. aureus* isolates all displayed beta hemolysis on blood agar. The tube coagulase test yielded negative results for 21 (32.18%) of 96 *staphylococcal* isolates. *S. aureus* was the most frequently isolated pathogen from Surf Field Mastitis test positive (sub-clinical) milk samples from two dairy farms, clinically mastitis milk samples from five dairy farms, and clinically mastitis milk samples submitted to Mastitis Research Lab. University of Agriculture Faisalabad. This study matches Ali *et al.* (2011), who examined 200 foremilk samples from 200 mastitic quarters of dairy buffalo. (clinically mastitis quarter n=17, sub-clinically n=183) for pathogen isolation. Under clinical was determined by Surf Field Mastitis Test (SFMT). Mastitis pathogens (n=107) obtained from subclinical (Surf field Mastitis Test positive) animals of two study herds and clinical mastitic milk samples submitted to Mastitis Research Lab, University of Agriculture, Faisalabad.

Table 4. Frequency of different pathogen recovered from clinical mastitis samples from positive samples Submitted to Mastitis Research Lab University of Agriculture Faisalabad.

Pathogen	No. of isolates	Percentage
Staphylococcus Aureus	43	41.21
Staphylococci(Cagulase -ve)	23	19.21
Coryneacterium Species	15	5.75
Coliforms	9	6.54

*Zam Zam Dairy Farm, Irfan Dairy Farm, Nazir Dairy Farm, Bilal Dairy Farm, Arz-e-Pak Dairy Farm in district Faisalabad.

Figure 1. Growth of *Staphylococcus aureus* isolates on blood agar plates.

Figure 2. Growth on MacConkey agar plate (yellow colonies non- lactose fermenting colonies, red colonies Lactose fermenting colonies).

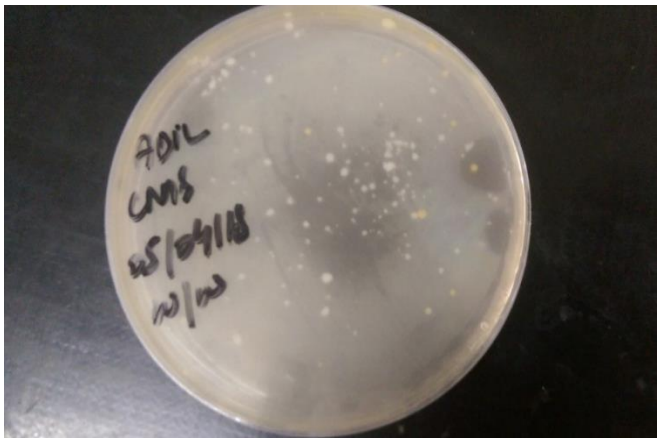


Figure 3. Colonies of bacteria on nutrient agar plates for colony counting.

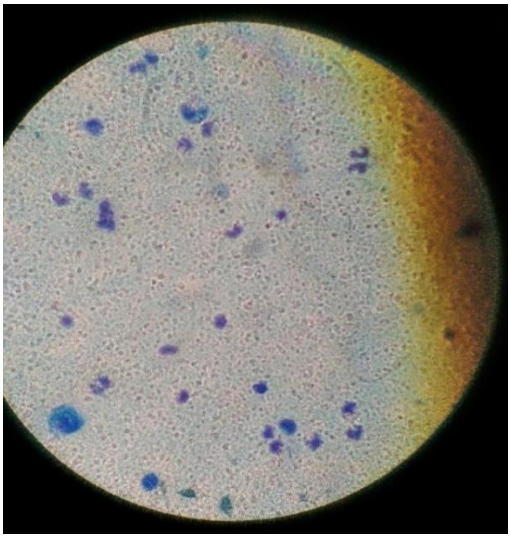


Figure 4. Micrograph of somatic cell count.

Somatic cell count: The value of (P=0.015) is less than (P≤0.05) so, it is significant. It reveals that by adopting hygienic measures, pre-dipping and post dipping procedures, there is a significant decrease in somatic cell count as mentioned in figure 5.

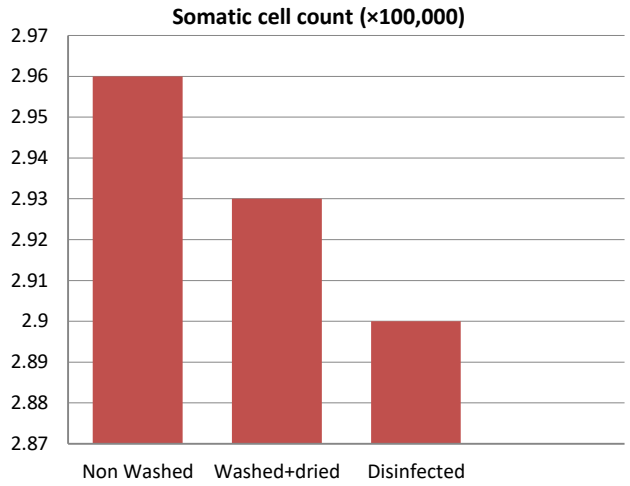


Figure 5. Bar chart of somatic cell count of Zam Zam Dairy Farm.

The value of (P=0.011) is less than (P≤0.05) so, it is significant. It reveals that by adopting hygienic measures and pre-dipping and post dipping procedures, there is a significant decrease in somatic cell count and bacterial load in milk as mentioned in Figure 6.

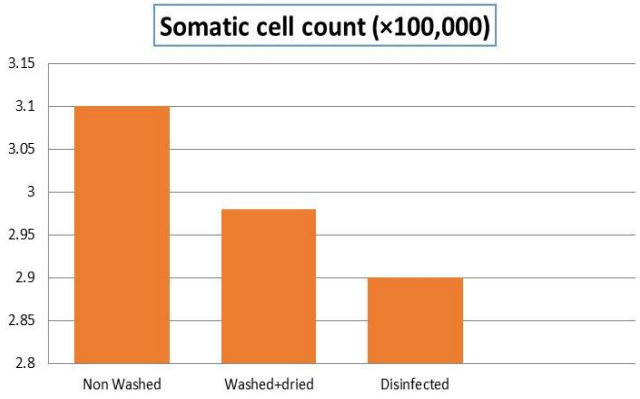


Figure 6. Bar chart of somatic cell count of Irfan Dairy Farm.

The value of (P=0.00) is less than (P≤0.05) so, the value is highly significant. It reveals that by adopting hygienic measures and pre-dipping and post dipping procedures. There is a reduction in somatic cell count and bacterial load in milk as mentioned in figure 7.

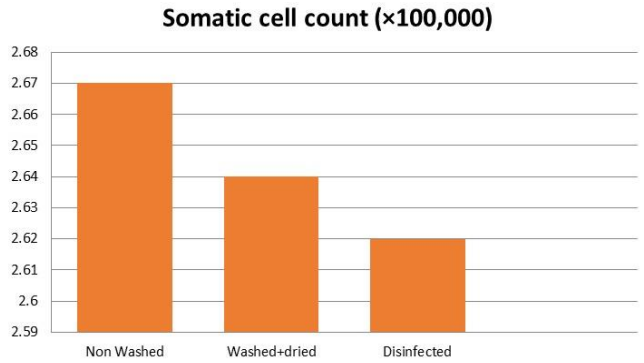


Figure 7. Bar chart of somatic cell count of Nazir Dairy Farm.

The value of (P=0.017) is less than (P≤0.05) so, the value is highly significant. It reveals that by adopting hygienic measures and pre-dipping and post dipping procedures. There is a decrease in somatic cell count and total bacterial count in milk as mentioned in figure 8.

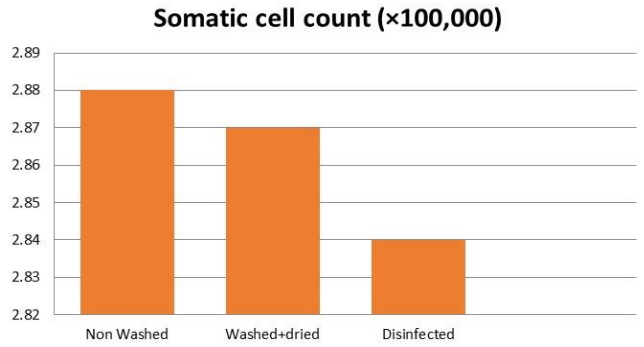


Figure 8. Bar chart of somatic cell count of Bilal Dairy Farm.

The value of (P=0.017) is less than (P≤0.05) so, the value of "p" is highly significant. It reveals that by adopting hygienic measures and pre-dipping and Post dipping procedures. There is a decrease in somatic cell count and total bacterial count in milk as mentioned in figure 9.

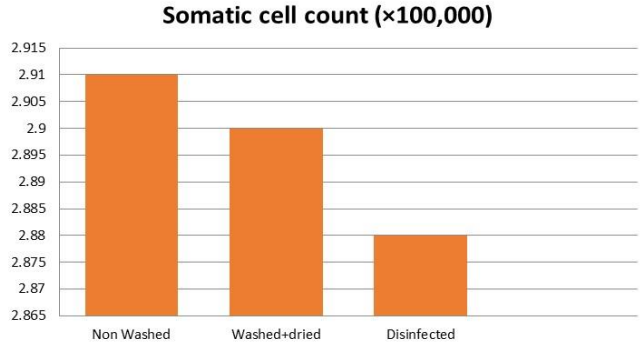


Figure 9. Bar chart of somatic cell count of Arz-e-Pak Dairy Farm.

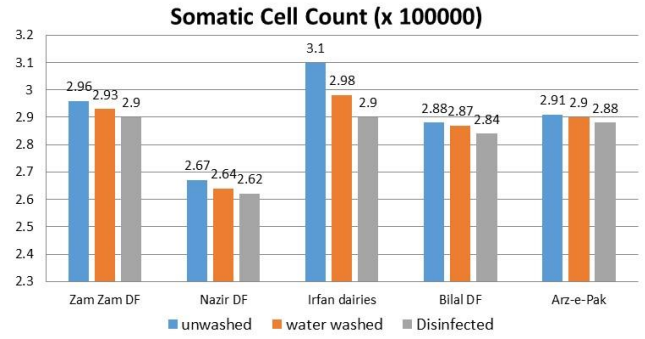


Figure 10. Mean somatic cell count of three groups of five dairy Farms.

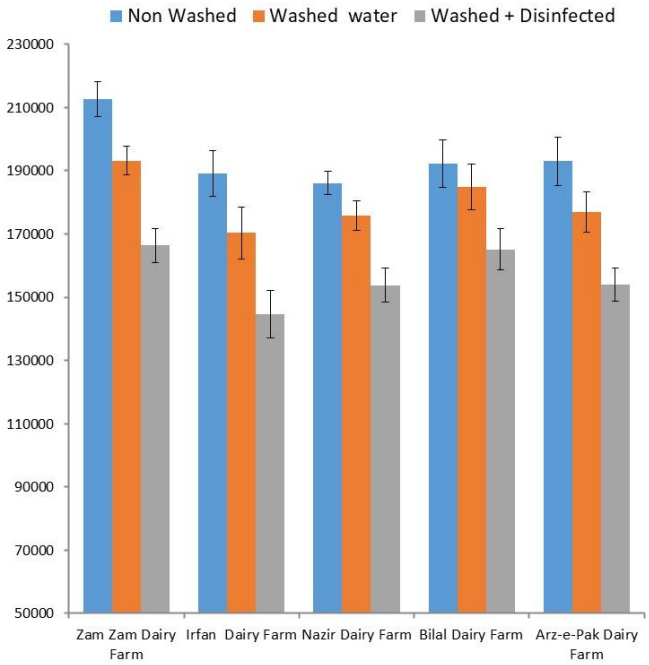


Figure 11. Mean somatic cell count of three groups of five dairy Farms.

***Colony count*:** It is a method that is used for the calculation of bacterial count in raw milk. The milk as such is not used for microbial count. Then the milk is diluted tenfold by serial dilution. Ten test tubes were taken having nine ml of distilled water in each one ml milk sample in the first tube and then mixed it gently take a sample from the first tube and put it into the second tube, mix it gently then so on do the same procedure at the tenth tube. It is better leave the first five tubes and then take a milk sample from 6^{th}, 7^{th} 8^{th}, 9^{th} or 10^{th} tube and pour it into nutrient agar incubate it for 24 hours and then calculate the number of colonies. The value of (P=0.022) is less than (P≤0.05) so, the value is significant. It reveals that in the first observation, there was no hygienic measure, so the value cfu/ml was higher. In the second observation only

Table 5. Comparison between none washed and washed with water and dried with respect to somatic cell count and colony count for different Dairy farms.

Dairy Farm	Character	Washing	N	Mean	SD	SE	t-value	P-value
ZamZam	Somatic cell count	Non-Washed	10	212600.00	17716.53	5602.46	2.69*	0.015
		Washed water	10	193220.00	14314.93	4526.78		
	Colony count	Non-Washed	10	3.55	0.91	0.29	1.67	0.113
		Washed water	10	2.92	0.75	0.24		
Irfan	Somatic cell count	Non-Washed	10	189130.00	23159.26	7323.60	1.70	0.106
		Washed water	10	170340.00	26070.98	8244.37		
	Colony count	Non Washed	10	3.05	0.95	0.30	1.41	0.175
		Washed water	10	2.51	0.75	0.24		
Nazir	Somatic cell count	Non Washed	10	186050.00	11649.06	3683.76	1.75	0.097
		Washed water	10	175740.00	14546.80	4600.10		
	Colony count	Non Washed	10	3.14	0.56	0.18	1.63	0.119
		Washed water	10	2.72	0.59	0.19		
Bilal	Somatic cell count	Non Washed	10	192220.00	23690.35	7491.55	0.71	0.484
		Washed water	10	184770.00	22914.00	7246.04		
	Colony count	Non Washed	10	3.37	0.55	0.17	1.27	0.221
		Washed water	10	3.05	0.58	0.18		
Arz-e-Pak Dairy Farm	Somatic cell count	Non Washed	10	192960.00	24303.19	7685.34	1.61	0.124
		Washed water	10	176870.00	20140.29	6368.92		
	Colony count	Non Washed	10	3.73	0.67	0.21	2.10	0.050
		Washed water	10	3.16	0.53	0.17		

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

washing on cleaning of the udder was performed so, the number of cfu/ml was less than in the first observation. In the third observation there was proper washing, cleaning, and disinfection of the udder was done so the number of cfu/ml was less as compared to both previous groups as mentioned in Figure 12.

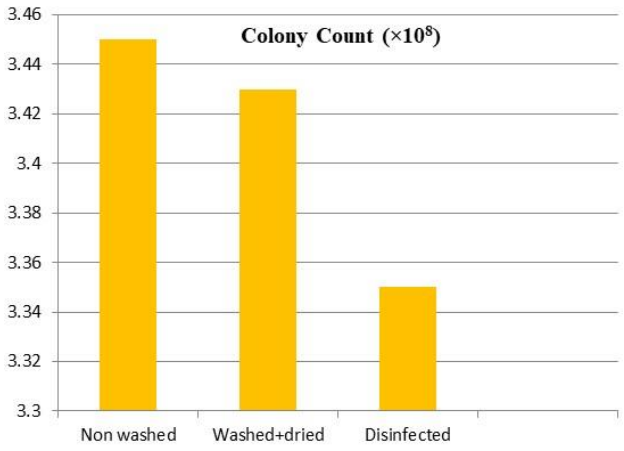


Figure 12. Bar chart of colony count of Zam Zam Dairy Farm.

The value of (P=0.001) is less than (P≤0.05) so, the value of "p" is significant. It reveals that in the first observation, there was no hygienic measure, so the value cfu/ml was higher. In the second observation only washing on cleaning of the udder was performed so, the no of cfu/ml was less than in the first observation. In the third observation there was proper washing, cleaning, and disinfection of the udder was done so the number of cfu/ml was less as compared to both previous groups as mentioned in Figure 13.

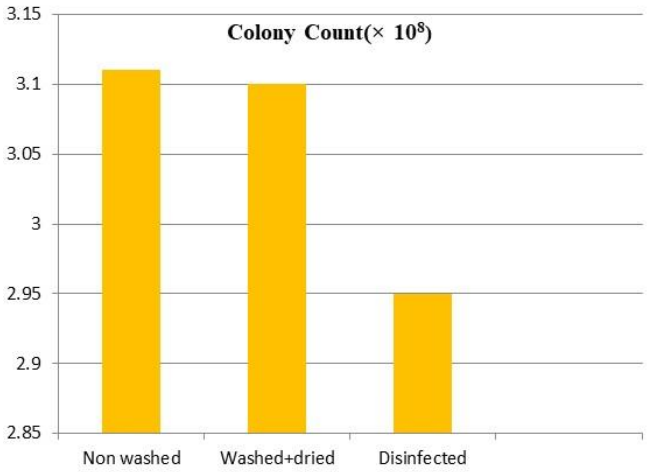


Figure 13. Bar chart of colony count Irfan Dairy Farm.

The value of (P=0.001) is less than (P≤0.05) so, so the value of "p" is significant. It reveals that in the first observation,

there was no hygienic measure, so the value cfu/ml was higher. In the second observation only washing on cleaning of the udder was performed so, the nunmber of cfu/ml was less than the first observation. In the third observation there was proper washing, cleaning, and disinfection of the udder was done so the number of cfu/ml was less as compared to both previous groups as mentioned in Figure 14.

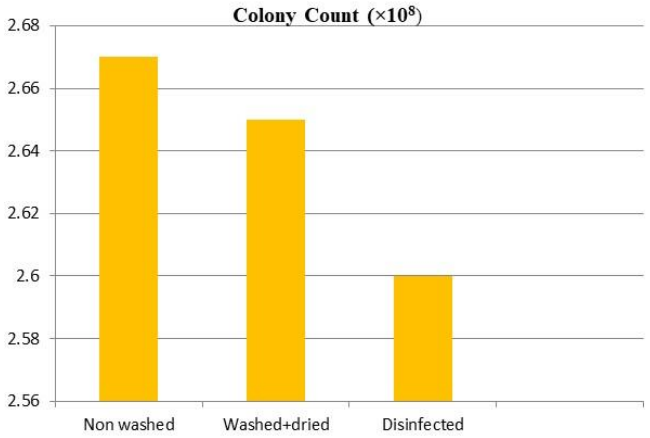


Figure 14. Bar chart of colony count Nzair Dairy Farm.

The value of (P=0.001) is less than (P≤0.05) so the value of "p" is significant. It reveals that in the first observation, there was no hygienic measure, so the value of cfu/ml was higher. In the second observation only washing on cleaning of the udder was performed so, the number of cfu/ml was less than in the first observation. In the third observation there was proper washing, cleaning, and disinfection of the udder was done so the number of cfu/ml was less as compared to both previous groups as mentioned in Figure 15.

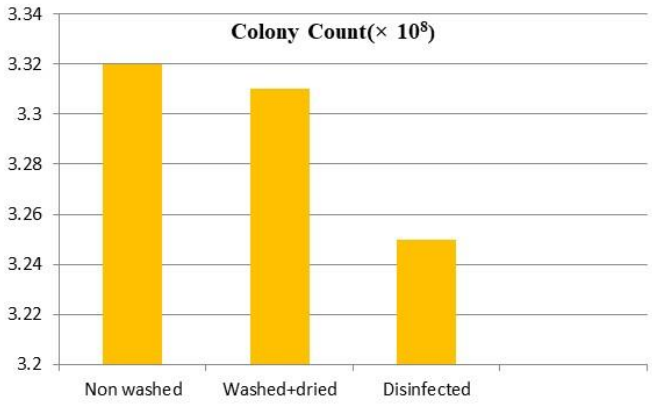


Figure 15. Bar chart of colony count Bilal Dairy Farm.

The value of (P=0.00) is less than (P≤0.05) so the value of "p" is significant. It has been shown in the first group, there was no hygienic measure, so the value cfu/ml was higher. In the second observation only washing on cleaning of the udder was performed so, the number of cfu/ml was less than in the first observation. In the third observation there was proper washing, cleaning, and disinfection of the udder was done so the number of cfu/ml was less as compared to both previous groups as mentioned in Figure 16.

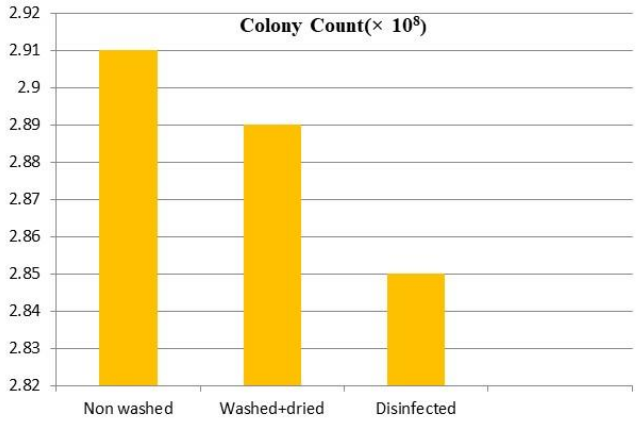


Figure 16. Bar chart of colony count Arz-e- Pak Dairy Farm.

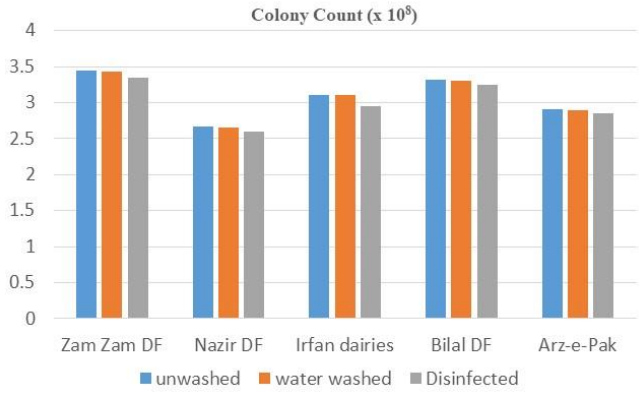


Figure 17. Mean of colony count of three groups of five dairy Farms.

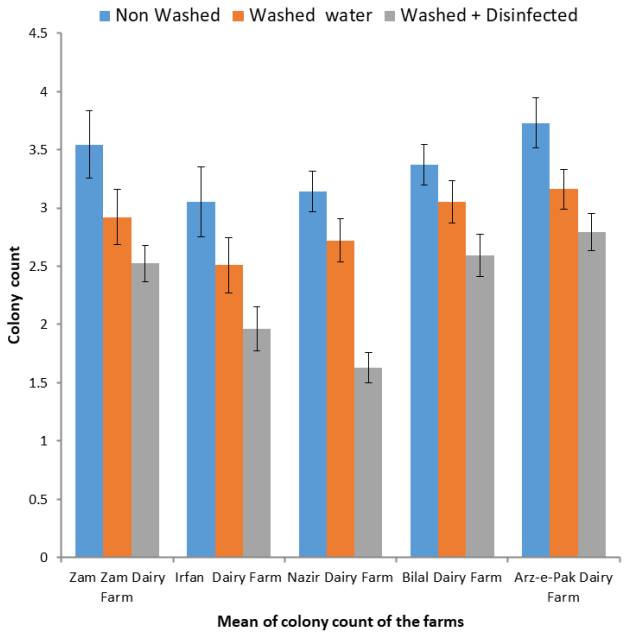


Figure 18. Mean of colony count of three groups of five dairy Farms.

DISCUSSION

Somatic cells are the main indicators for diagnosis of udder infection. In the healthy udder, the normal range of somatic cell count is 275,000 cells/ml (Hillerton, 1999). Moreover, somatic cell count also has a correlation with the quality of milk. According to Table 1, the floor and surface area were not even. There was an accumulation of urine. The body of animals was soiled with fecal material, mostly the hind quarter of the animal. When in such conditions milking is being done. The somatic cell count, and bacterial count become high. According to Capuco *et al.* (1992) at the time of milking, when microbes are present near the teat orifice, after milking they enter into the teat orifice, which remains open from half an hour to one hour, produce disruption and injury to the fibrous protein (keratin) or epithelial membranes in the teat canal. Then, they not only affect the milk quality but also cause mastitis. It was observed that there was a significant difference (P>0.05) in the somatic cell count between commercial farms and non-commercial farms. According to Suranindyah *et al.* (2015), environmental conditions have a significant effect on clean milk production and in mastitis control. It was observed that the body of animals was very dirty. The fecal material adheres to the body of animal as well as to the udder of animal. Then, it leads to the deterioration of the milk quality. At the time of milking the dung or fecal material adhering to skin of udder may fall into bucket. If there is accumulation of dung near the milking area, then milk will absorb the fecal smell. Because milk is excellent absorber. According to Muhammad *et al.* (2010), the milk produced under unhygienic condition, from dirty animals and without washing of udder has high level of somatic cell count. Poor milk practices also increased SCC and degraded the milk quality. The unclean udder or soiled with fecal material also increased the SCC count in the milk. The milk having high somatic cell count is not only suitable human consumption in raw form. But, also for processing milk products. For certain results, the test should be performed in pre-milking, after provoking the milk let down and discarding the first few strips of foremilk. The overall prevalence in five herds (mentioned above in Table 2) was 44.90 %(n=167out377) animals effected with clinical (n=6 buffaloes=12 cows) and sub-clinical mastitis. The overall subclinical mastitis in cows in these farms was 61%. While the similar value for buffalo was 32%. According to Swami *et al.* (2017) subclinical mastitis prevalence is 21.7% of 60 normal healthy animals and 8% of foremilk samples. Most studies were done on cattle and buffaloes. By providing similar managemental practices, there is more occurrence of mastitis in cattle as compared to buffaloes. The prevalence of clinically positive and sub-clinical positive animals in Indian Punjab was 7.8% and 48% (Ali *et al.*, 2011).

conducted a study to evaluate the effect of subclinical mastitis on the chemical properties of milk In both cattle and buffalo pH of milk of subclinical mastitis increased than normal milk Swami *et al.* (2017). The fat, protein, SNF, and lactose percentage decreased significantly. Subclinical mastitis is sometimes also due to factors like, age, lactation no., stage of lactation, method of milking and housing management all are the risk factors contributing in subclinical mastitis. In India histological work has been done revealing that there is lesser susceptibility of buffalo mastitis as compared to cow. This may be due to well-developed smooth muscles around the teat orifice in this spp. In buffaloes, the mastitis defense is stronger as compared to cattle due to unique morphology of the teat. The epithelium of the skin of buffalo teat is compact compared to cattle and has more resistance against mastitis. Moreover, the keratin layer present in the teat canal is thicker in buffaloes. The most important thing about keratin is that it has bacteriostatic and bactericidal activity. The cationic protein is inhibitory to the growth of *streptococcus agalactiae* and *staphylococcus aureus*. The entry of microorganisms into the mammary gland almost invariable occurs *via* the teat canal. Although the mechanism by which bacteria cross the teat canal are not yet fully understood. De Silva *et al.* (2016) suggested that there is strong evidence that invasion of the mammary gland is controlled largely by the characteristics of the teat canal. Another, reason for the lesser susceptibility of buffalo to mastitis may be the preponderance of neutrophils in the uninfected mammary gland as opposed to macrophage in the uninfected mammary gland of dairy cows as per De Silva *et al.* (2016), for the phagocytosis and destruction of invading pathogen, large number of neutrophils are available

in buffalo milk. The neutrophils are more preferably the more efficient phagocytic cells.

The Prevalence of subclinical mastitis was assessed in a population of dairy buffaloes raised under organized, small holdings and individual holdings farming conditions in Narowal, Lahore, Okara and Sahiwal using the White Side Test (WST). The prevalence was higher at individual-holding buffalo farms (52%) as compared with small holdings (48%) and organized (32%) farms. Differences may be due to poor hygiene and managemental conditions. The cumulative percentage of mastitis in buffaloes observed by WST was 44% which is much lower than that of 92% recorded by Cinar *et al.* (2015). Similarly, an incidence of 54.7, 32.85,31.9% and 23.18% has been reported by De Silva *et al.* (2016). Variations in the prevalence of mastitis might be due to the different regions, therapeutic practices, management conditions, and presence of microorganisms in the environment.

The somatic cell count and colony count are lower in washed udder as compared to non-washed udder. According to Ruud *et al.* (2011) fecal material adhering to the skin of udder and teat contain various pathogens that contaminate the milk. After washing of udder, the fecal material is removed. While normal microflora present on the skin of teat is also reduced to some extent. That's why the leukocyte count is reduced. The udder management and cleanliness is very important for clean milk production. Moreover, the deterioration of milk quality the unhygienic conditions cause clinical and sub-clinical mastitis. Chishty *et al.* (2007) studied that mastitis is considered one of the deadly disease economically and the occurrence of mastitis is due to unhygienic conditions. The quantity of milk is reduced drastically in mastitis. According to Rao (1990) due to mastitis, there is a change in the physico-chemical properties. The pH of mastitis milk is increased from the normal milk which ultimately reduces the shelf life of the milk. Moreover, the enteric pathogen present in the milk is contagious and cause severe foodborne diseases in humans like *Tuberculosis*, *Salmonellosis*, and *Leptospirosis*, is zoonotic in nature. The colony count was high in the group of dirty animal. While the value of colony count was lower in disinfected udder, it has been shown that in each group the value of SCC as well as colony count is higher in unhygienic groups of animals. Similarly, in other group, where hygienic measures are adopted, the value of colony count is lower. The group of animal in which pre-milking and post-milking dipping was performed. There was significant P<0.05) decline in microbial load. According to Cinar *et al.* (2015) post-milking dipping play very important role in prevention of udder infection. After r the milking the teat orifice remains open for 15-30 minutes. There are number bacteria present at the bedding area. When the animal sit within 30 minutes there may be chance of udder infection. Among all the pathogens *Escherichia-Coli* is 75% more responsible in causing udder infection and deterioration of milk quality. Post milking dipping is very effective germicide and known as important element in prevention n of udder infection (Contreras *et al.*, 2007).

According to Allore (1993) animal surroundings such as bedding and manure are source of common contagious pathogens. These pathogens may be present in soil and air as environmental microorganisms. Milker's hands, cloth towels and flies spread these pathogenic bacteria to clean udders during the milking process and are responsible for most of the clinical infection

According to Ruud *et al.* (2011) the fecal material adhering to hides contains enteric bacteria, possibly pathogenic, thus representing a potential source of food-borne disease. Additionally, cattle cleanliness affects hygienic milk production, thermoregulation, health conditions, and hide quality.

***Conclusion*:** The present study emphasizes the importance of udder care and modern dairy procedures in preventing mastitis and producing clean milk. Proper udder disinfection considerably (P<0.01) reduced microbial contamination compared to basic water washing and non-washed udder, as measured by SCC and CC analysis. Results indicated that a decrease in SCC and CC in the disinfected and clean with towel udder significantly (P<0.01). However, SCC and CC were increased significantly (P<0.05) in control groups. All in all, by applying udder washing with disinfectant and cleaning with towel warranted the clean milk production. Pre- and post-milking dipping, udder cleaning, and milker hand hygiene reduced mastitis and enhanced milk quality on farms. Thus, contemporary dairy methods and strong cleanliness measures are vital for preventing mastitis and producing high-quality, safe milk.

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***Conflict of interest*:** N/A

***Ethical statement*:**

***Acknowledgement*:** N/A

***Informed consent*:** N/A

***Consent to participate*:** N/A

***Consent for publication*:** N/A

***SDGs addressed*:** Good Health and Well-being, Zero Hunger, Clean Water and Sanitation, Responsible Consumption and Production

***Policy referred*:**1. National Policy,"Policy referred: Pakistan Livestock Policy 2006,Option 2: International Standard ,"Policy referred: Codex Alimentarius – Milk and Milk Products (CAC/RCP 57-2004), Option 3: FAO/WHO Guidelines, "Policy referred: FAO/WHO Guidelines on

Hygienic Milk Production.Option 4: National Mastitis Control Program (NMCP) or Guidelines (if available in Pakistan).

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